(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 August 2004 (19.08.2004)

PCT

(10) International Publication Number
WO 2004/069265 A1

(51) International Patent Classification[7]: A61K 38/01, A23J 3/34, A23L 1/305, C12P 21/06, C12S 3/24, A61P 3/04

(21) International Application Number:
PCT/NL2003/000084

(22) International Filing Date: 7 February 2003 (07.02.2003)

(25) Filing Language: English

(26) Publication Language: English

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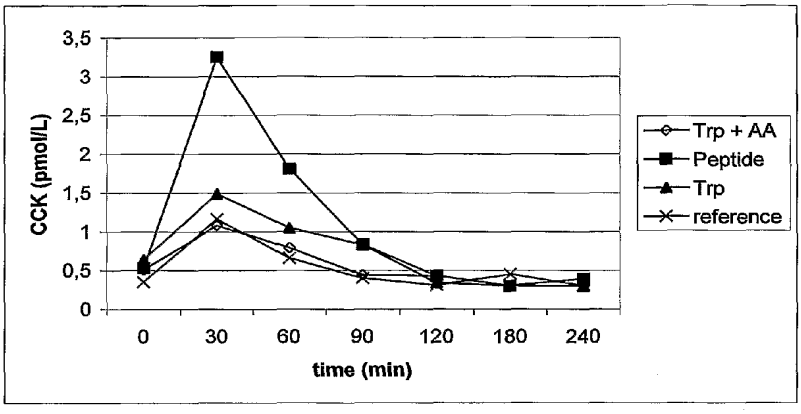
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF TRYPTOPHAN RICH PEPTIDES



(57) Abstract: Described is the novel use of peptides derived from whey protein hydrolysate as active ingredient in a medicament or as food ingredient for elevating the cholecystokinin level in the blood, and for preventing or treatment of overweight and/or obesity, in an animal, including human, in need thereof.

WO 2004/069265 A1

PATENT COOPERATION TREATY

PCT

REC'D 20 OCT 2003
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INTERNATIONAL SEARCH REPORT

3 of 12/2004

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
A03-40016/RKI		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 03/ 00084	07/02/2003	
Applicant		
CAMPINA B.V.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

USE OF TRYPTOPHAN RICH PEPTIDES

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[X] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

USE OF TRYPTOPHAN RICH PEPTIDES

The invention relates to a novel use of peptides derived from a whey protein hydrolysate.

In the art, several attempts have been made to elevate the cholecystokinin (CCK) levels in the intestine, e.g. by providing specially designed nutritive agents that are said to stimulate the release of CCK. Such a nutritive agent is described in US patent application US2002/0119915, wherein a powder composition is disclosed, comprising proteins, fatty acids and a proteinase inhibitor, that is to be ingested before a meal to extend post meal satiety. The proteinase inhibitor was described to be critical for the stimulation of CCK release. Although whey protein could be used as protein source in the said composition, peptides derived from a whey protein hydrolysate were not disclosed. Moreover, the presence of a proteinase inhibitor would prevent the formation of a hydrolysate.

It has now been surprisingly found that peptides derived from a whey protein hydrolysate have a positive effect in elevating the CCK level in an animal, including humans, in particular in the blood. CCK is known to play an important role in the treatment and prevention of obesity and overweight, by mediating a satiety signal in the animal (see e.g. A. Stafleu, Leads in Life Sciences, 2002, (14) pp. 9-10).

Therefore, the invention provides a novel use of peptides, derived from a whey protein hydrolysate, as active ingredient in a medicament or as food ingredient for elevating the cholecystokinin level in the blood of an animal, including human, in need thereof, and also for preventing or treatment of overweight and/or obesity.

The term "peptides" is known in the art; herein the term relates to amino acid chains, preferably having a molecular weight of 500-5000 Dalton, more preferably between 1000-3000 Dalton. It is e.g. general knowledge that proteins can be fragmented by hydrolysis into peptides that consist of a small number of amino acids.

The peptides for use according to the present invention are obtained by hydrolysis of whey protein, more preferably by enzymatic cleavage of a whey protein. Hydrolysis and enzymatic cleavage of

proteins to obtain peptides, i.e. protein fragments, are known techniques in the art.

In a preferred embodiment, the peptides are prepared by cleaving the whey protein by one or more acid proteases or cysteine proteases, preferably chosen from the group, consisting of pepsin, papain or bromelain, or a mixture of two or more thereof. Preferably, the protein source is cleaved by pepsin, preferably at a pH of between 1,5-3,5, more preferably between 2-3.

The peptides are derived from whey protein. It is observed that whey proteins have a relatively high tryptophan content (about 1,8 w/w%). In a very attractive embodiment of the invention, the peptides are derived from whey protein isolate, preferably whey protein concentrate, most preferably from α-lactalbumin enriched whey protein concentrate (WPC) or α-lactalbumin enriched whey protein isolate (WPI). The terms "whey protein isolates" and "whey protein concentrates" are known in the field, see e.g. Walstra et al., 1999, Dairy Technology, ISBN 0-8247-0228-X. Whey protein concentrate is a whey protein product having 35-80 w/w% protein, whereas whey protein isolate has a protein content of 90 w/w% or higher. An example of WPC is Lacprodan 80 from ARLA, Denmark; an example of WPI is Bipro from Bio-isolates Ltd. α Lactalbumin enriched whey protein isolates and concentrates are derived from whey protein and have an elevated α-lactalbumin content. The α-lactalbumin content of αWPC may e.g. vary, depending on the preparation, between 20-80 w/w%, whereas the αlactalbumin content of normal WPC is about 12-18 w/w%. α-Lactalbumin has a high tryptophan content of about 5,8w/w%. A whey protein isolate containing about 60w/w% α-lactalbumin can be obtained from DMV International, the Netherlands, and is described in EP 0 604 684.

In a preferred use according to the invention, the peptides are obtained by an isolation method, the said isolation comprising the steps of:

a) providing an aqueous whey protein hydrolysate,
b) controlling the pH of the aequeous whey protein hydrolysate to 4,0-6,0, forming a peptide precipitate, and
c) isolation of the precipitated peptides.

As outlined above, the skilled person is aware of suitable conditions for performing hydrolysis reactions on the whey protein. The term "controlling" of the pH means that the pH should be adjusted or kept at the above described pH value during the precipitation of the peptides.

Isolation of the precipitated peptides can be done by methods that are known in the art. The precipitated peptides can e.g. be collected by centrifugation, decantation or filtration and the like. In order to obtain a long shelf life, the isolation preferably comprises a drying step. The skilled person is aware of suitable drying techniques. As will be shown in the examples it has been found that the precipitated peptides, are effective in elevating the CCK level and can be used against overweight and obesity.

Preferably, the precipitation is carried out at a temperature below 20°C. Below said temperature, the peptides have shown to precipitate very efficiently.

As outlined above, the aqueous peptide mixture, i.e. the whey protein hydrolysate is preferably prepared by enzymatic cleavage of whey protein, and more preferably, the whey protein is cleaved at acidic pH by one or more acid proteases or cysteine proteases, especially by one or more enzymes, chosen from the group, consisting of pepsine, rennin, acid fungal proteases, chymosin, papain, bromelain, chymopapain or ficin or mixtures of two or more thereof. By cleavage of whey protein by one or more of said acid proteases, especially pepsin at a pH between 1,5 and 3,5, preferably between 2-3, peptides having a hydrophobic nature are generated. It was found that from these peptide mixtures, the effective peptides could very efficiently be selectively isolated by controlling the pH to 4,0-6,0, preferably to around 5,0. In case the pH at the enzymatic cleavage was below 4,0, the pH was to be adjusted to 4,0-6,0 in order to precipitate the peptides. Preferably, the enzymatic activity is quenched by inactivation of the enzyme before the precipitation step. The skilled person will know how to inactivate the proteolytic enzyme. In case an enzyme is chosen having its pH optimum within the above mentioned pH range of 4,5-6,0, such as e.g. papaine or bromelaine, it will be possible to design the isolation method in such way that cleavage of the whey protein and precipitation of the peptides can occur simultaneously. Care has to be taken that the

precipitation is done at conditions wherein the hydrolysed peptides preferentially precipitate; otherwise, a precipitate of partial hydrolysed peptides may be obtained.

Preferably, the peptide mixture is desalted before the step of controlling the pH (step b). It has been found that a desalting step prior to the pH controlling step leads to an improved yield of precipitated peptides. Desalting is a known technique and can be done by e.g. nanofiltration, ultrafiltration or electrodialysis. Especially when the peptides are obtained by enzymatic cleavage, desalting the obtained peptide mixture leads to improved yields. Desalting is preferably carried out such that 50-95% of the salt present during the cleavage reaction is removed from the peptide mixture.

By the above-described isolation method, a peptide mixture can be obtained, that can advantageously be used in e.g. a food ingredient or a medicament for the elevation of CCK levels and against obesity and overweight.

The invention further relates to a method for elevating the cholecystokinin level in the blood of an animal, including human, in need thereof, comprising the step of administering to the animal an effective amount of peptides from a whey protein hydrolysate as described above. The administration can be performed according to methods, known in the art; the peptide mixture can be administered as a medicament, comprising a suitable carrier. The administration route can be any route known in the art, such as, but not limited to oral percutaneous route. The medicament can be in any known form, such in the form of pills, ointments, or injection fluids. The peptide mixture can also be administered as a powder or be incorporated in a food product.

The invention also relates to a method for preventing or treatment of overweight and/or obesity of an animal, including human, in need thereof, comprising the step of administering to the animal an effective amount of peptides from a whey protein hydrolysate as described above.

The invention will now be further illustrated by some non-limiting examples and a figure, wherein the mean CCK concentrations in plasma of human volunteers (in pmol/l, n=8) is shown at several time points after consumption of peptides according to the invention

(black squares), of amino acids including tryptophan (blank diamonds), of tryptophan as amino acid (black triangle) and of a reference substance (cross).

The percentages in the examples are weight percentages, unless indicated otherwise.

Example 1

Preparation of peptides derived from whey protein hydrolysate

A whey protein isolate solution containing 75% α-lactalbumin (Davisco) is dissolved in demineralised water, resulting in a solution comprising 2,8 w/w% dry solids and 2 w/w% α-lactalbumin. The pH is adjusted to 2.0 using 2M phosphoric acid. Hereafter the said mixture is heated to 50°C.

The hydrolytic reaction is started by adding 0.5% E/S pepsin (Merck, USA). E/S stands for the enzyme/substrate ratio. After 6 hours the reaction is stopped by incubating the reaction for 10 minutes at 90°C. Subsequently, the pH was raised to 5.0 and the temperature was lowered to 4°C. After storage of 20 hours at this temperature, the precipitated peptides are collected by decantation and centrifugation and subsequent freeze drying.

Tryptophan is determined using a specific technique based on total enzymatic hydrolysis (Garcia, S.E.; Baxter, J.H. (1992) Determination of tryptophan content in infant formulas and medical nutrition. *J. AOAC Int.* 75:1112-1119). The amino acids phenylalanine, tyrosine, leucine, isoleucine, valine and methionine are determined according EG guideline 98/64 (3-9-1998; publication L257/14-23 of 19-9-1998). Protein (81,1%) is determined using the standard Kjeldahl method (IDF-FIL 20A, 1986). The resulting product contains 8,5% tryptophan on product, and 10.4% on peptide.

A chemical and amino acid analysis is given in table 1.

Table 1

Chemical analysis (expressed on powder)	
Protein (Kjeldalh N*6.38)	81.1%
Fat	3.7%
Lactose	< 0.1%
Ash (825°C)	4.8%
Amino Acid Analysis	
Tryptophan on powder (Trp)	8.5%
Trp/protein	10.4%
Trp/LNAA	0.37
(Large Neutral Amino Acids: Val, Tyr, Leu, Ile, Phe)	
Amino Acid Profile (per gram of protein):	
Alanine (Ala)	51.4 mg
Arginine (Arg)	7.3 mg
Aspartic acid (Asp)	96.8 mg
Cystine (Cys)	74.2 mg
Glutamic acid (Glu)	155.4 mg
Glycine (Gly)	14.9 mg
Histidine (His)	59.9 mg
Isoleucine (Ile)	25.3 mg
Leucine (Leu)	131.6 mg
Lysine (Lys)	109.6 mg
Methionine (Met)	4.9 mg
Phenylalanine (Phe)	55.4 mg
Proline (Pro)	63.0 mg
Serine (Ser)	47.6 mg
Threonine (Thr)	77.6 mg
Tryptophan (Trp)	104.3 mg
Tyrosine (Tyr)	22.4 mg
Valine (Val)	43.6 mg
Total:	1145.2 mg

Example 2

Preparation of peptides, derived from whey protein hydrolysate 2

A whey protein isolate (WPI), containing 60% α-lactalbumin (experimental product of DMV International, The Netherlands) is dissolved in an aqueous solution. The pH of the solution is adjusted using diluted phosphoric acid and heated to 45°C. Hydrolysis is started by adding 2% pepsin (Merck, 2500 FIP-U/g) and carried out for 2 hours. The reaction is stopped by pasteurising the solution at 85°C for 10 minutes. Hereafter, the pH is raised to 5.5

and the solution is cooled to <15°C. After 10 hours, the precipitated peptides are collected using microfiltration. Typically, a membrane having a nominal molecular weight cut-off of 1 µm is used. The peptides are hereafter spray dried. The resulting product contains 9.3% tryptophan on peptide.

Example 3

Preparation of peptides, derived from whey protein hydrolysate 3

A whey protein solution similar to reference example 1 was hydrolysed with pepsin (American Laboratories, USA) using enzyme/substrate ratios (E/S) in w/w% of 0.25% and 0.75%. After 5 hours, the reaction was stopped by raising the pH to 5.2 using 1.0M NaOH and cooling the solution to <15°C.
The precipitated peptides were harvested after 16 hours by centrifugation.

Example 4

Preparation of peptides derived from whey protein hydrolysate 4

A 10% whey protein solution containing 45% α-lactalbumin (DMV International, The Netherlands) was dissolved in demineralised water. The pH was adjusted to 7.0 using 1M sodium hydroxide. Hereafter the solution was heated to 50°C.
The hydrolytic reaction was started by adding 2% ENZECO Bromelain 240 (Enzyme Development Corporation). After 21 hours the reaction was stopped by heating the solution to 85°C for 10 minutes. Subsequently, the peptide mixture was cooled to room temperature, the pH adjusted to 4.5 using phosphoric acid and the temperature is lowered to 10°C. After storage during 12 hours at this temperature, the precipitated peptides were collected by centrifugation and subsequent freeze drying.
The resulting tryptophan content of the peptides was 8%.

Example 5

Preparation of peptides derived from whey protein hydrolysate 5

100 l of a 5% whey protein isolate solution (Davisco) was prepared and then hydrolysed using 2% Pepsin. The solution was hydrolysed for

12 hours at pH 3.0. The reaction was stopped by heating the solution to 80°C for 30 minutes. Hereafter, the solution was ultrafiltrated on a pilot NF unit using Celgard NF-PES-10 membrane. The pH of the retentate was controlled at 3.0 and the solution filtered up to 200% diafiltration.

After desalting, the pH of the retentate was adjusted to 5.5 and the solution is cooled to <10°C to facilitate precipitation of the envisaged peptides. After 10 hours of storage, the precipitate was collected using centrifugation. Hereafter, the peptides were dried.

The tryptophan and peptide concentration in the sample was 9.5% and 91%, respectively.

Example 6

Increase of CCK levels on ingestion of tryptophan rich peptides

The experiment described below was performed in a double-blind, four period, randomised, cross-over, placebo controlled study.

Eight healthy human volunteers were divided into four groups of two, and were refrained from any food overnight. In the morning, the test persons obtained orange juice (containing 25 g glucose) and possibly a test substance, as follows:

Group 1: orange juice containing 5,91 g peptides as obtained in example 1 per single dose orange juice (200 ml)
Group 2: orange juice containing 500 mg pure tryptophan (Ajinomoto USA, Inc.)
Group 3: orange juice containing a mix of free amino acids in the identical composition and concentration as in the juice of group 1. The said amino acids were purchased from Ajinomoto USA, Inc.)
Group 4: orange juice without any test substance.

The experiment was repeated four times such, that all the eight volunteers eventually obtained aal the four test substances. During the four hours following the ingestion of the orange juice, blood was taken from the test persons at t=0, 30, 60 90, 120, 180 and 240 minutes after ingestion. CCK analysis was carried out using a

radio-immunoassay (RIA) kit of Euro-Diagnostica (cat nr. #RB302) according to the instructions of the manufacturer.

The results are shown in table 2 below and in figure 3, showing a maximum CCK level of 3.25 pmol/l at t=30 minutes after ingestion. The basal level in humans is normally about 1 pmol/l and increases to between 3 and 8 pmol/l after a meal (Becker et al., Am. J. Surg., 1984 (147) pp. 124-129). As the maximum level of CCK is gradually reached between 10 and 45 minutes after ingestion of a meal (Himeno, Am. J. Gastroenterol., 1983 (78) pp. 703-707), it is very well possible that the maximum CCK level is higher than 3.25 pmol/l, and occurring between t=0 and t=30 minutes. The increasing plasma levels with 2-4 pmol are deemed to be relevant in increasing perception of satiety.

TABLE 2

Mean CCK levels in plasma (in pmol/l, n=8)

CCK (pmol/L)	amino acids	Trp-peptide	Tryptophan	Control
0 minutes	0.51±0.60	0.53±0.65	0.64±0.78	0.35±0.15
30 minutes	1.08±0.87	3.25±1.48	1.49±1.04	1.16±1.31
60 minutes	0.79±0.86	1.81±0.90	1.05±1.06	0.66±0.67
90 minutes	0.44±0.29	0.83±0.89	0.83±0.84	0.40±0.18
120 minutes	0.43±0.38	0.43±0.36	0.34±0.11	0.31±0.04
180 minutes	0.30±0.00	0.30±0.00	0.30±0.00	0.45±0.43
240 minutes	0.30±0.00	0.39±0.25	0.30±0.00	0.30±0.00

CLAIMS

1. Use of peptides, derived form a whey protein hydrolysate as active ingredient in a medicament or as food ingredient for elevating the cholecystokinin level in the blood of an animal, including human, in need thereof.

2. Use of peptides, derived from a whey protein hydrolysate as active ingredient in a medicament or as food ingredient for preventing or treatment of overweight and/or obesity in an animal, including human, in need thereof.

3. Method according to any of the preceding claims, wherein the peptides are prepared by enzymatic cleavage of whey protein.

4. Method according to claim 3, wherein peptides are prepared by cleaving the whey protein by one or more acid proteases or cysteine proteases, preferably chosen from the group, consisting of pepsin, papain or bromelain, or a mixture of two or more thereof.

5. Method according to claim 4, wherein the whey protein is cleaved by pepsin at a pH of between 1,5-3,5, preferably between 2-3.

6. Use according to any of the preceding claims, wherein the peptides are derived from whey protein isolate.

7. Use according to any of the preceding claims 1-5, wherein the peptides are derived from whey protein concentrate.

8. Use according to any of the proceeding claims, wherein the peptides are derived from α-lactalbumin enriched whey protein.

9. Use according to any of the preceding claims, wherein the peptides are obtained by an isolation method, the said isolation method comprising the steps of:

a) providing an aqueous whey protein hydrolysate,

b) controlling the pH of the aqueous whey protein hydrolysate to 4,0-6,0, forming a peptide precipitate, and

c) isolation of the precipitated peptides.

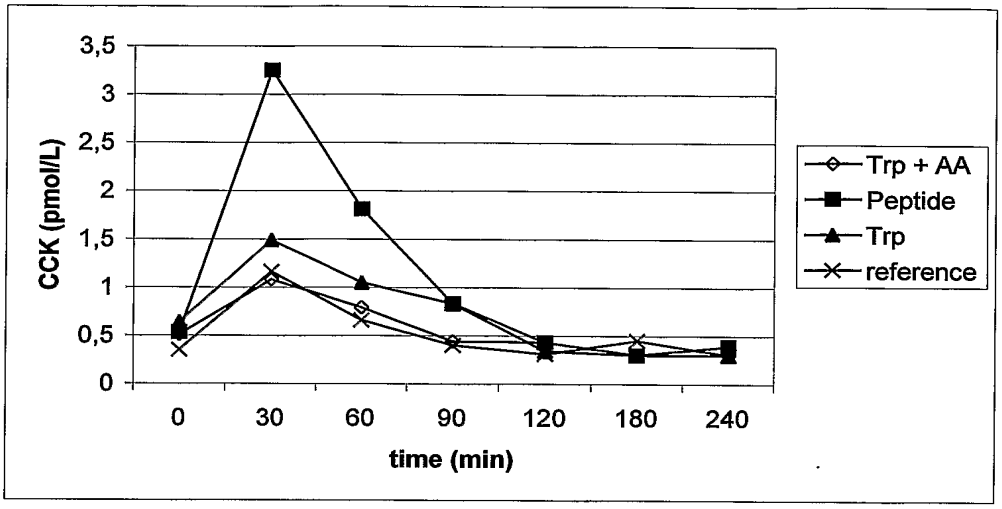
10. Use according to claim 9, wherein step a) is carried out at a temperature of below 20°C.

11. Use according to any of the preceding claims, wherein the peptides are desalted.

12. Method for elevating the cholecystokinin level in the blood of an animal, including human, in need thereof, comprising the step of administering to the animal an effective amount of peptides derived form a whey protein hydrolysate as defined in any of the claims 1-11.

13. Method for preventing or treatment of overweight and/or obesity of an animal, including human, in need thereof, comprising the step of administering to the animal an effective amount of a peptides derived from a whey protein hydrolysate as defined in any of the claims 1-11.

FIG. 1


3,5
3
2,5
2
1,5
1
0,5
0
CCK (pmol/L)
0
30
60
90
120
180
240
time (min)
Trp + AA
Peptide
Trp
reference

INTERNATIONAL SEARCH REPORT

PCT/NL 03/00084

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/01 A23J3/34 A23L1/305 C12P21/06 C12S3/24
A61P3/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AOYAMA T ET AL: "EFFECT OF SOY AND MILK WHEY PROTEIN ISOLATES AND THEIR HYDROLYSATES ON WEIGHT REDUCTION IN GENETICALLY OBESE MICE" BIOSCIENCE BIOTECHNOLOGY BIOCHEMISTRY, JAPAN SOC. FOR BIOSCIENCE, BIOTECHNOLOGY AND AGROCHEM. TOKYO, JP, vol. 64, no. 12, December 2000 (2000-12), pages 2594-2600, XP001109501 ISSN: 0916-8451	2,13
Y	the whole document	1-13
Y	WO 02 46210 A (CAMPINA MELKUNIE BV ;BOUMANS JOHANNES WILHELMUS LEO (NL); CAESSENS) 13 June 2002 (2002-06-13) page 5, line 36 -page 6, line 5; claims 1-13	1-13

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
9 October 2003	22/10/2003

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

PCT/NL 03/00084

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	G.A. BRAY: "Reciprocal relation of food intake and sympathetic activity: experimental observations and clinical implications." INTERNATIONAL JOURNAL OF OBESITY, vol. 24, no. suppl. 2, 2000, pages S8-S17, XP002257146 page S8, abstract table 1 ---	1-13
Y	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 7 March 2001 (2001-03-07) MAHER TIMOTHY J ET AL: "The serotonin precursor 5-hydroxy-L-tryptophan decreases food intake in food-deprived and stressed rats" Database accession no. PREV200100243535 XP002257148 abstract & FASEB JOURNAL, vol. 15, no. 4, 7 March 2001 (2001-03-07), page A223 Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biol;Orlando, Florida, USA; March 31-April 04, 2001 ISSN: 0892-6638 ---	1-13
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A	WO 87 01590 A (KREITZMAN STEPHEN NEIL) 26 March 1987 (1987-03-26) claims ---	1-13
A	WO 99 52363 A (NUTRICEUTICAL TECHNOLOGY CORP ;UNIV NEW YORK (US); WHITE JEFFREY () 21 October 1999 (1999-10-21) claims 1,2 ---	1-13

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 429 190 B1 (PORTMAN ROBERT) 6 August 2002 (2002-08-06) column 1, paragraph 1; claims column 5, line 66 -column 6, line 11	1-13

INTERNATIONAL SEARCH REPORT

PCT/NL 03/00084

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12 and 13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/NL 03/00084

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